

<p>(1390 REV. 5-93) US DEPT. OF COMMERCE PATENT &amp; TRADEMARK OFFICE</p> <p><b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b></p>		<p>ATTORNEY'S DOCKET NUMBER 112387</p> <p>U.S. APPLICATION NO. (if known, sec 37 C.F.R.1.5)</p> <p style="font-size: 1.5em; font-weight: bold;">10/089835</p>
<p>INTERNATIONAL APPLICATION NO. PCT/JP00/06979</p>	<p>INTERNATIONAL FILING DATE October 6, 2000</p>	<p>PRIORITY DATE CLAIMED October 7, 1999</p>
<p>TITLE OF INVENTION A METHOD OF TESTING FOR HEPATIC CIRRHOSIS USING A BREATH ANALYSIS APPARATUS AND THE APPARATUS</p>		
<p>APPLICANT(S) FOR DO/EO/US Kouichi ISHIKAWA, Yukimoto ISHII, Satoshi ASAI, Kazuo NAKANO, Keiji HASUMI</p>		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p>		
<p>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</p> <p>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p style="margin-left: 20px;">a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</p> <p style="margin-left: 20px;">b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</p> <p style="margin-left: 20px;">c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</p> <p>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p style="margin-left: 20px;">a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> have been transmitted by the International Bureau.</p> <p style="margin-left: 20px;">c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p style="margin-left: 20px;">d. <input type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</p> <p><b>Items 11. to 16. below concern other document(s) or information included:</b></p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input type="checkbox"/> A FIRST preliminary amendment.</p> <p style="margin-left: 20px;"><input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> Entitlement to small entity status is hereby asserted.</p> <p>16. <input type="checkbox"/> Other items or information:</p>		

(1390 Rev.10-00)

2/pv

A METHOD OF TESTING FOR HEPATIC CIRRHOSIS USING A BREATH  
ANALYSIS APPARATUS AND THE APPARATUS

TECHNICAL FIELD

The present invention relates to a method of testing for hepatic diseases and a breath analyzing apparatus used for the method.

BACKGROUND ART

Diagnosis of hepatic diseases is performed by a clinical view by a doctor, a liver biopsy, a ventroscopy, liver scanning, a ultrasonic examination, CT scanning, X-ray inspection, or the like.

However, since these methods need a special technique by a doctor, a special engineer, or the like, and expensive equipment, they are not suitable for the purpose of testing for hepatic diseases in a general medical checkup or the like.

For this reason, in a medical checkup, the hepatic diseases is examined by extracting blood and urine and analyzing metabolites in the blood and the urine.

As examination of such hepatic diseases, there are methods of measuring of blood serum bilirubin, ZTT, TTT, ALP, CHE, GOT and GPT, gamma-GTP, LDH, LAP, blood serum total protein, a A/G ratio, urine bilirubin, urine urobilinogen, or the like. When it is indicated in such test that the subject may suffer from hepatic diseases, he undergoes further examination and analysis ~~as mentioned above in a medical~~ institution. On the other hand, in recent years, there is proposed a method of testing for various disorders by measuring metabolites in breath. Such a method is described,

for example in Yasuhiro Mitsui, "detection system of trace components in breath", bulletin of S14-5 Showa 62 National Convention of Institute of Electrical Engineers of Japan (1987)).

However, a test for hepatic diseases by measuring metabolites in blood and urine has a disadvantages that it takes much time to obtain a result. Therefore, it may be suitable for neither monitoring of a patient in a medical institution, nor testing in the case of urgent hospitalization. Moreover, blood collecting can be conducted only by a person having a certain qualification, and furthermore, it inflicts a pain on the patient, which causes a problem especially if the patient is in a serious condition or a child. Furthermore, the above-mentioned measurement data of metabolites in blood and urine is not always specific for hepatic diseases. Therefore, in order to perform the most exact diagnosis, it is important to obtain as many measurement data as possible and to make diagnosis with a combination of those data. There has not been disclosed a specific method of using the above-mentioned breath analysis for testing for hepatic diseases.

An object of the present invention is to provide a method of testing for hepatic diseases which enables a quick judgment, inflicts little pain on a patient, and can provide an exact judgment, and an apparatus used for the method.

## DISCLOSURE OF THE INVENTION

In order to achieve the above-mentioned object, the inventors of the present invention have aimed at breath analysis which inflicts almost no pain on a patient, and have studied thoroughly about the relation between components in

breath and hepatic diseases, and thereby have completed the present invention.

(1) The present invention relates to a method of testing for hepatic diseases comprising collecting breath, quantifying isopropanol and/or cyanides in the breath, and analyzing a result thereof.

(2) The present invention also relates to the above testing method for testing for hepatic cirrhosis.

Furthermore, the present invention relates to the following breath analyzing apparatuses for testing hepatic diseases.

(3) A breath analyzing apparatus for testing hepatic diseases comprising a breath collecting section for introducing breath to be analyzed, a breath analyzing section wherein isopropanol and/or cyanides in the breath are quantified, and a data-processing section which analyzes the measured result obtained by the breath analyzing section.

(4) The breath analyzing apparatus described in (3) wherein the breath collecting section consists of a breath collecting means and a breath transfer means.

(5) The breath analyzing apparatus described in (4) wherein the breath collecting means is a mouthpiece or a mask.

(6) The breath analyzing apparatus described in (4) wherein the breath collecting means is a communicating opening for connecting a breath container.

(7) The breath analyzing apparatus described in any one of (4) to (6) wherein the breath transfer means comprises a duct which connects the breath collecting means and the breath analyzing section so that the breath can flow through them.

[illegible]

(8) The breath analyzing apparatus described in (7) wherein the breath transfer means includes further a pump means to send breath to the breath analyzing section.

(9) The breath analyzing apparatus described in (7) or (8) wherein the breath collecting means includes both the mouthpiece or the mask and the communicating opening for connecting the breath container, and a valve which can be switched so that only one of them which is chosen depending on the case can be communicate with the breath analyzing section is provided at the duct.

(10) The breath analyzing apparatus described in any one of (3) to (9) wherein the breath analyzing section comprises a mass spectrometer.

(11) The breath analyzing apparatus described in any one of (3) to (10) used for test for hepatic cirrhosis.

The present invention also provides the above-mentioned methods and the above-mentioned apparatuses wherein the cyanides in breath is quantified by quantifying HCN which is a decomposition product thereof.

### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic view showing a breath analyzing apparatus of an embodiment of the present invention.

Fig. 2 is a graph showing the relation between hepatic cirrhosis and each of an amount of isopropanol and an amount of cyanides.

Fig. 3 is a schematic view showing a breath analyzing apparatus of another embodiment of the present invention.

Fig. 4 is a schematic view showing a breath analyzing apparatus of another embodiment of the present invention.











healthy person significantly differs from those of a hepatic disease patient. Thus, a judging method suitable for the test actually conducted can be adopted by collecting the data of quantification of isopropanol and a cyanide in breath of, for example, six healthy persons and the same number of hepatic disease patients.

## 2. Breath Analyzing apparatus

As to the apparatus of the present invention, the breath collecting section is a section for collecting the breath to be analyzed, introducing it into the apparatus, and leading it to the breath analyzing section. It preferably consists of a breath collecting means for collecting breath, and a breath transfer means for transporting the collected breath to the breath analyzing section.

The breath collecting means may be, for example, a breath blowing-in opening such as a mouthpiece for collecting breath directly, a mask in the form which covers a mouth or both a nose and a mouth, a communicating opening to which a breath container is connected; or the like.

When the test is performed by introducing breath into the apparatus directly, the above-mentioned blowing-in opening is used. When the test is performed by collecting breath in a breath container and introducing it into the apparatus for quantification after a certain time, the above-mentioned communicating opening is used.

The breath container is a container for collecting the breath to be analyzed. Examples thereof include: a glassware like a vacuum bottle, a breath collecting bag made of a synthetic resin, for example, the products made of elasticity

vinyl chloride, vinyl tetrafluoride, tetraethylene fluoride, and a polyethylene phthalate and the like.

The above-mentioned mouthpiece and mask may have a structure which can collect breath efficiently without leaking it outside. Furthermore, it is desirable to provide a means for preventing blown-in breath from leaking to the open air, for example, a valve.

The breath transfer means may include, for example, a duct which connects the breath collecting means and the breath analyzing section so that breath may pass through, a valve provided in the duct, a pump for sending or introducing breath to the breath analyzing section compulsorily.

In order to suppress adsorption of the substance to be tested in breath to the duct, the inside of the duct is desirably subjected to electropolishing. Furthermore, for the same purpose as the above, the apparatus of the present invention is equipped with a heating means for heating a breath collecting section, especially the duct and the valve, to a constant temperature.

Furthermore, the breath transfer means may include a breath amount control mechanism. It is desirable to have a structure which makes it possible to send a certain amount of the collected breath to the breath analyzing section.

Moreover, in the breath collecting section, there may be provided a pretreatment means for subjecting the breath to pretreatment such as concentration, absorption to a solution, adsorption, condensation, elimination of impurities or moisture with a filter, separation by gas chromatography or the like.

In the present invention, the breath analyzing section

is a section wherein isopropanol and/or cyanides in breath is quantified, and this section includes a quantitative-analysis instrument which can quantify these substances.

Specific examples of the quantitative-analysis instrument may include: a mass spectrometer, a spectrographic-analysis meter, a fluorometric-analysis meter, gas-chromatograph equipment (gas-solid chromatograph equipment, gas-liquid chromatograph equipment), liquid chromatograph equipment, an indicator tube, a semiconductor sensor, IR analyzer (for example, FT-IR), an ion-electrode concentration measuring apparatus, a photoelectric photometer, the colorimeter, and the like. Preferable examples of the mass spectrometer include: an electronic ionization mass spectrometer, a chemical ionization mass spectrometer, an atmospheric-pressure-ionization-mass-spectrometry meter, a secondary ion mass spectrometer, a fast-atom-bombardment ionization mass spectrometer, a thermospray ionization mass spectrometer, an electro spray ionization mass spectrometer, a laser desorption ionization mass spectrometer, or the like. The separation of ions may be for example, a magnetic field single convergence type, an electric-field magnetic field double-focusing type, a quadrupole type, a 3-dimensional quadrupole type, a TOF type, and an ICR type. Moreover, GC-MS equipment, MS-MS equipment, LC-MS equipment, or the like can be used.

In the present invention, the "data-processing section" is a section where judgment as for the hepatic diseases is carried out by analyzing the result obtained by the breath analyzing section. If desired, there are also performed in the section calculation of concentration of isopropanol and/or



the scope of the present invention. The skilled in the art may carry out various improvement and modification based on the description of the present invention and information known in the art. In all drawings showing the examples, the same numerals are used for the parts having the same function respectively, and same explanation is not repeated for those parts.

### Example 1

Fig.1 shows a schematic view of the breath analyzing apparatus of this example.

The apparatus for testing for hepatic cirrhosis of this Example, that is a breath analyzing apparatus 1a consists of a breath collecting section 28, a breath analyzing section 29, and a data-processing section 30 as shown in Fig. 1. In the breath collecting section 28, a mouthpiece 2 and a breath collecting bag 6 are connected to a three-way valve 5 by an duct 52. Switch valves 3 and 4 provided in the valve 5 is used for switching between the case where breath is directly introduced from the mouthpiece and the case where breath is indirectly introduced after once collected in the breath collecting bag 6. The above-mentioned valve 5 is connected to a breath introduction opening of the breath analyzing section 29 via a flow controller 7, and controls a flow of the breath introduced directly or indirectly to a certain value, and introduces it into the breath analyzing section 29.

A duct of which inner surface is subjected to electropolishing is used as the duct constituting the breath collecting section 28, and the duct and the valve 5 are heated to a constant temperature by a heater 8. Thereby, adsorption

[illegible]

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Fig.3 shows a schematic view of the breath analyzing apparatus 1b of this example. In this example, an ion trap mass spectrometer 34 is used in a breath analyzing section. Since the ion trap mass spectrometer 34 is an analyzer which makes microanalysis possible, as in the case of APIMS, the substance to be analyzed in breath can be analyzed with high sensitivity. The ion trap mass spectrometer 34 consists of an ionization section 31 and a high-vacuum section 32. There is provided an ionization means by electric discharge or the like in the ionization section 31, which ionizes the breath introduced from the breath collecting section 28. The high-vacuum section 32 is the section maintained at high vacuum by an evacuation system 36. An ion trap electrode 33 and a detector 35 are provided therein. Ions generated in the ionization section 31 are subjected to trap concentration, which are then detected by the detector 35. Then, they are converted to an electrical signal and transmitted.

As described above, according to this Example, a simple test for hepatic cirrhosis can be conducted by analyzing cyanides and isopropanol in breath using the breath analyzing apparatus with the ion trap mass spectrometer 34.

Fig.4 shows a schematic view of the breath analyzing

apparatus 1c of this example. In this example, a gas-chromatograph mass spectrometer 42 is used in a breath analyzing section. Since both a qualitative analysis and a quantitative analysis can be conducted simultaneously by the gas-chromatograph mass spectrometer 42, breath analysis can be performed without identifying a peak in advance. The gas-chromatograph mass spectrometer 42 consists of a carrier gas introducing section, a column 37, an interface 38, and a mass spectrometer 39. In the carrier gas introducing section, the carrier gas cylinder 43 is connected with the column 37 via the reducing valve 44 and the flow controller 45. Thereby, the carrier gas can be supplied to a column 37 at a certain pressure and at a certain flow. In the column 37, the substances are separated by difference in chemisorption of the substance. The interface 38 connects the mass spectrometer 39 to the column 37, and controls a gas flow, measurement timing or the like. The mass spectrometer 39 is maintained at a high vacuum by the evacuation system 40, and the separated ions are detected by the detector 41, converted to an electrical signal, and then transmitted.

Operation of this Example will be explained below. The carrier gas maintained at a certain pressure by the reducing valve 44 and maintained at a certain flow by the flow controller 45 is introduced into the column 37 with the breath introduced by the breath collecting section 28. The substance to be analyzed in the introduced breath is introduced into the mass spectrometer 39 through the interface 38, after being separated by feature of the substance. In the mass spectrometer 39, they are ionized and separated, then detected by the signal detector 41, and converted to electrical signal

and transmitted. According to this Example, a simple test for hepatic cirrhosis can be conducted by analyzing cyanides and isopropanol in breath using the breath analyzing apparatus with the gas-chromatograph mass spectrometer 42.

#### INDUSTRIAL APPLICABILITY

According to a test method of the hepatic diseases of the present invention and the breath analyzing apparatus therefor, a test for hepatic diseases can be conducted without requiring an engineer having special technology, without giving a subject pain, and the results can be obtained immediately.

Moreover, a still more exact judgment of hepatic diseases can be achieved by combining with other test results.

Therefore, a simple, exact, quick test for hepatic diseases can be conducted not only at medical institutions, such as a hospital, but also at a medical checkup center or a health center.

Furthermore, the apparatus of the present invention makes a teletherapy such as monitoring of a person recuperated at home in a remote place or the like possible.









SECRET

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FIG.1

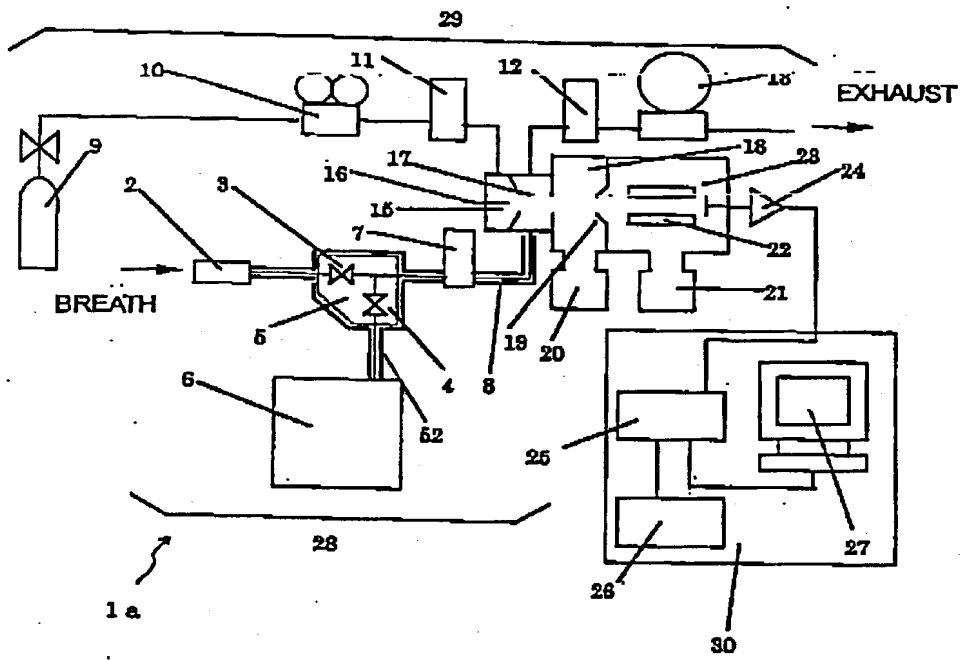
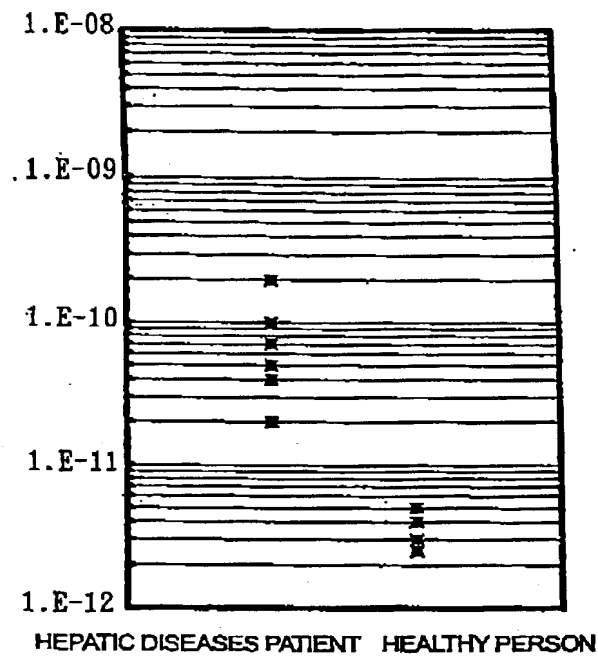
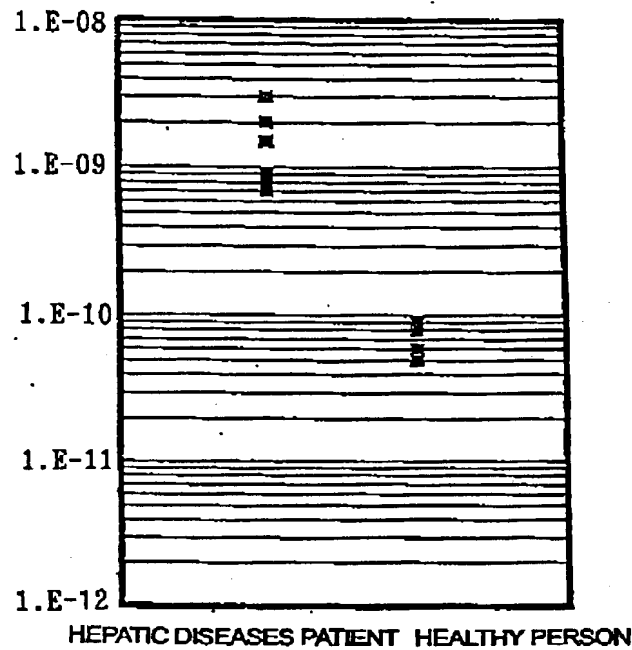


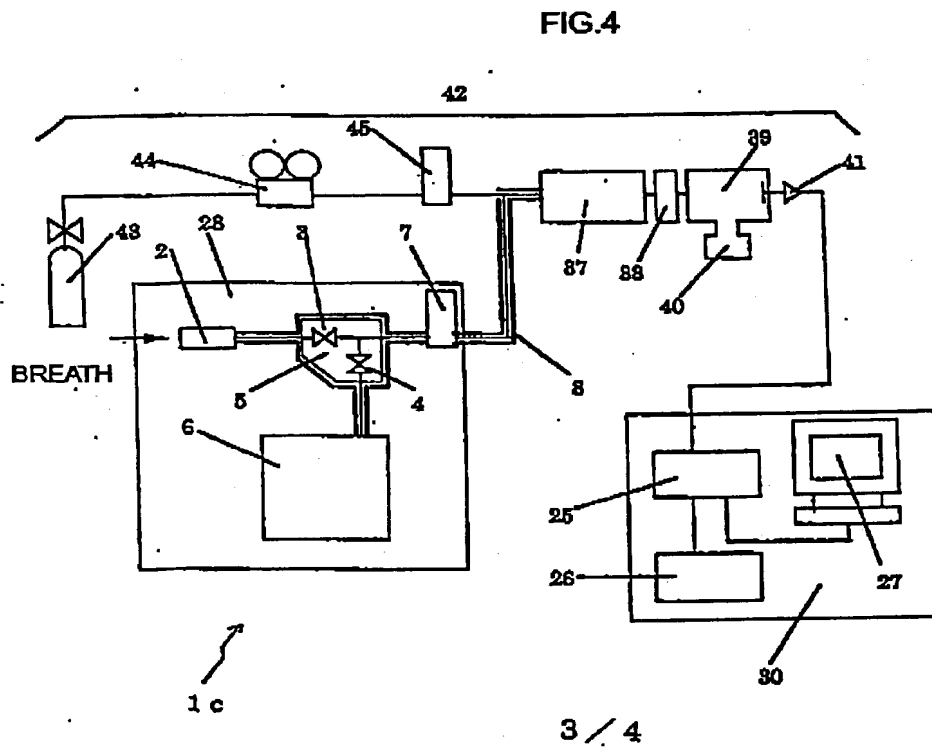
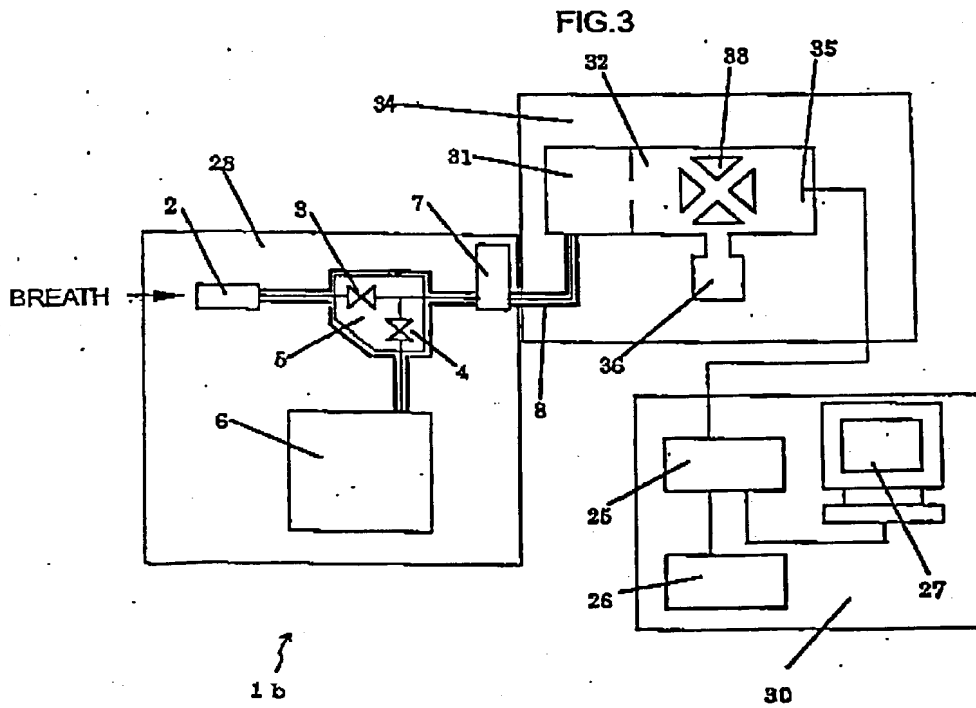
FIG.2



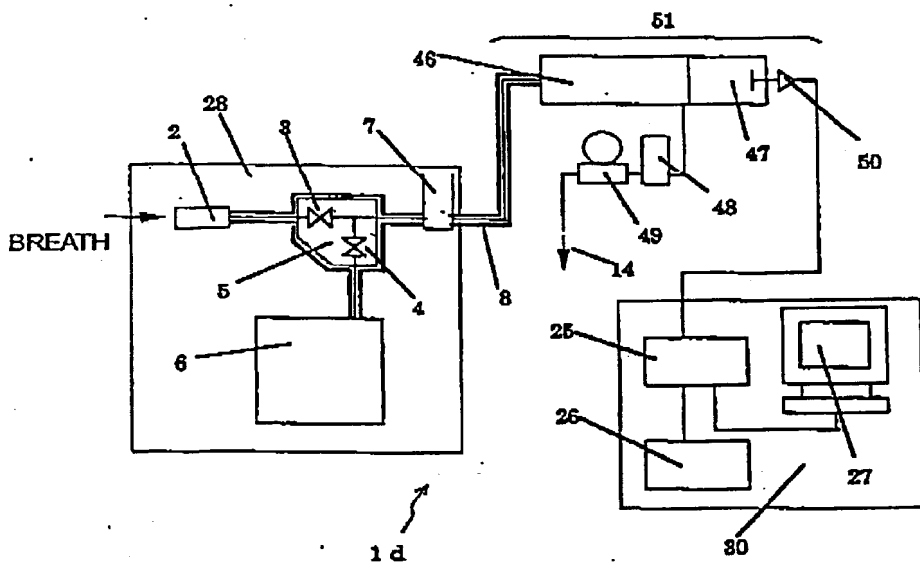
(A)



(B)



**FIG.5**



**DECLARATION AND POWER OF ATTORNEY  
UNDER 35 USC §371(c)(4) FOR  
PCT APPLICATION FOR UNITED STATES PATENT**

As a below named inventor, I hereby declare that:

my residence, post office address and citizenship are as stated below under my name;

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, namely the invention entitled: A METHOD OF TESTING FOR HEPATIC CIRRHOSIS USING A BREATH ANALYSIS APPARATUS AND THE APPARATUS

described and claimed in international application number PCT/JP00/06979 filed October 6, 2000.

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) filed by me or my legal representatives or assigns within one year prior to my international application are hereby claimed:

Japanese Patent Application No. 11-286335 filed October 7, 1999

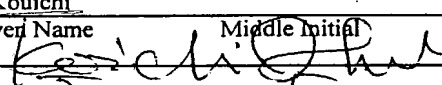
The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to my international application, or (b) before the filing date of the above-named foreign priority application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

James A. Oliff, Reg. No. 27,075; William P. Berridge, Reg. No. 30,024;  
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ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE, PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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IF THERE IS MORE THAN ONE INVENTOR USE PAGE 2 AND PLACE AN "X" HERE ☒  
(Discard this page in a sole inventor application)

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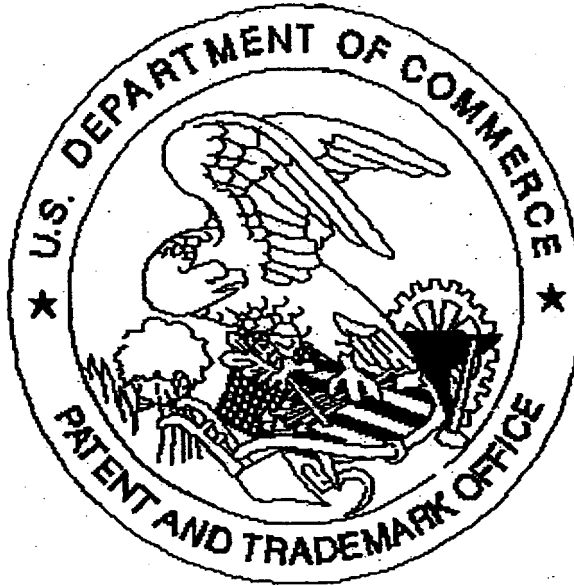
c/o Hitachi Tokyo Electronics Co., Ltd.

610-5, Shimoimasuwa, Shirane-cho, Nakakoma-gun, Yamanashi 400-0212, Japan

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